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Paper: Fetal radiofrequency radiation exposure from 800-1900 MHz-rated cellular telephones affects neurodevelopment and behavior in mice.

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Introduction: There is an extensive body of literature describing the effects of prenatal exposure of rodents and other animals to radiofrequency (RF) fields (e.g. ICNIRP 2009). Overall, these studies indicate that exposures to RF fields do not cause any consistent effect on behaviour of offspring in the absence of maternal hyperthermia. The thresholds for effects from RF-induced hyperthermia are comparable with those induced by other forms of heating (Edwards et al, 2003). In an extensive study, Bornhausen and Scheingraber (2000) reported that continuous exposure of rats to low level GSM 900 MHz cellular phone signals during pregnancy had no effect on a the performance of a battery of operant behavioural tasks by offspring at 3 months of age. In addition, no interaction mechanism has been established whereby exposure to RF fields in the absence of heating could cause teratological effects.

Few studies have examined whether cellular phone use may affect the development of children. An epidemiology study (Divan et al, 2008) has reported that prenatal and postnatal use of cellular telephones by mothers in Denmark is associated emotional and hyperactivity problems around the age of school entry. However, a follow-up study (Divan et al, 2011) found no evidence of an association between prenatal cell phone use and developmental milestone delays among infants at 6 and 18 months of age.

As a model for effects in children, Aldad and coworkers investigated the effects of in utero RF field exposure from cellular phones. The performance of three behavioural tasks examining working memory, anxiety and activity were investigated in young adult mice, and electrophysiological recordings of the neuronal activity in the prefrontal cortex of young mice were made to help identify underlying physical causes.

Methods: The exposure system used in this study was very simple. An ordinary cellular phone was suspended over each of the animal cages such that the animals were between 4.5 cm and 22.3 cm from the cellular phone, depending on their location within their cage. The phone was called from a landline in order to ensure that it was radiating. Control exposure was achieved using a phone that was deactivated. The animals were exposed for 24 h per day for 17 days (behavioural tests) and 9, 15 or 24 h per day for 17 days (electrophysiology). Three female mice were exposed concurrently in each cage; a male was introduced into each cage at the beginning of the experiment, but it is not stated when these were removed.

The behaviour of the offspring was examined using three behavioural tasks. Memory was examined using an object recognition task that utilizes a rodent's preference to explore previously unseen (novel) objects. Animals were allowed to explore two identical objects for 15 min per day for two

days. On the third day, one of these objects was replaced with a new object and the exploration of these objects for 2 min was analysed. Memory was assessed using a preference index (time of exploration of new object/combined time of exploration of both objects, multiplied by 100). These tests were performed when the animals were 8, 12 or 16 weeks old.

Activity and anxiety were investigated using a box consisting of an illuminated compartment and a dark compartment. The number of crossings between the compartments was measured to analyse activity, and anxiety by measuring the time spent in each compartment. These tests were performed when the animals were 12, 15 or 18 weeks old.

Fear was assessed using a step-down test. The time it takes an animal to get down from a small raised platform was recorded: increased fear was associated with a greater time to step-down. These tests were performed when the animals were 12 or 40 weeks old.

Lastly, coronal brain slices from the prefrontal cortex were made when the animals were 3-4 weeks old Patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) were made in pyramidal neurons in cortical layer V.

Results: In the memory task, the preference index of the exposed animals was less than that of the controls for each time point (mean of 56.8%, 69.4% and 63.5% in the exposed group compared to 66.5%, 71.7% and 71.2% in the control group, at 8, 12 and 16 weeks old respectively). No statistical analysis of these data is reported. The greatest difference occurred at 8 weeks with less difference at 16 weeks and only marginal difference at 12 weeks. The cumulative mean preference index of the exposed animals for all three time points (63%) was significantly lower than that of the combined sham exposed group (69.9%). This lower preference was taken as suggesting that exposed had caused impairment in memory. The amount of time both treatment groups spent not exploring the objects were not significantly different (between 90 and 97% for all time periods).

In the activity and anxiety task, the mean number of crossings between compartments was increased at each time point for the exposed group compared to the control group (mean of 29.9, 32.5 and 14.8 compared to 23.9, 13.8 and 6.5 in the control group, at 12, 15 and 18 weeks old respectively). These differences were said to be significant but no statistics were provided. The cumulative mean number of crossings in the exposed group was 24.4 while that of the control group was 16.4: this difference was statistically significant, suggesting exposure increased activity. The mean times the exposed group of animals spent in the dark compartment were less than the control group for all time periods (210.8, 187.0 and 235.8s compared to 225.6, 215.5 and 270.6s in the control group at 12, 15 and 18 weeks respectively). No statistics on these data are presented. However, the exposed animals spent a cumulative mean of 207s in the dark compared to a mean of 234s for the control group; this difference was statistically significant, suggesting that exposure resulted in decreased anxiety.

In the step down task, there were no obvious differences between treatment groups at either time point in mean times on the platform. Again, only statistical analysis of the cumulative mean times was performed; these were not statistically different at either 12 or 40 weeks. This suggested that exposure did not affect fearfulness.

Patch clamp recordings made in cortical layer V pyramidal neurons indicated that exposure resulted in a significant decrease in the frequency of mEPSCs (0.72 ± 0.06 Hz in exposed animals compared to 1.00 ± 0.12 Hz in controls) and in a significant decrease in the amplitude of the mEPSCs. These

results were taken as evidence that exposure had impaired glutamatergic transmission at both pre- and postsynaptic sites. When the effects of different exposure durations per day were examined, it was found that the reduction in mEPSCs frequency was dependent on hours per day exposed; the greatest decrease was seen with 24 h exposures, 15 h caused slight reductions in mEPSCs frequency while 9 h per day was comparable to control. Overall, a linear dose response relationship was seen between the decrease in mEPSCs frequency and exposure time per day. No changes in mEPSCs frequency were reported in neurons of the ventral medial hypothalamus, although a decrease in mEPSCs amplitude was seen. This suggested that the changes in neuronal function were not limited to one area of the cortex.

Discussion: Using cellular phones as an exposure system is not suitable or appropriate for animal experiments, as the exposures cannot be controlled or described with any certainty. The extent of the dosimetry is that the active phone is described as having a specific energy absorption rate (SAR) level of 1.6 W/kg, but this is the phone's rating when running at full power, as measured using a phantom human head in the tissues closest to the device. Without active power control the phone's emissions would not have been stable or predictable, and could be at a level very much less than those that would give rise to a SAR of 1.6 W/kg even if the phone was against the head. The paper also mentions that an additional six females were exposed to an active phone for either 9 or 15 hours per day. However, if the phone had been running at full power for these lengths of time then the battery would have run out of power. This suggests that the phones should have been powered by a charger; but this is not discussed in the paper.

At 4.5–22.3 cm, the SAR in a human head would be very small indeed: at the extreme end of the distance range it would be a few mW/kg at most, and could be at μW/kg levels when the lack of power control of the phone is taken into account. Furthermore, the RF absorption cross section of a mouse is quite different than that of a human head; the dimensions of mice are a small fraction of a wavelength in the frequency range 900 – 1900 MHz and even with their bodies aligned with the electric field component of an RF signal they will be much weaker RF absorbers than a human head.

Having more than one free-moving animal in the cage during exposure further complicates the dosimetry, as the activity and behaviour of the animals will influence the absorbed power experienced by each. Taken together, the lack of power control, the distance between source and animals and the small absorption cross section of mice suggest that exposure levels of exposed animals may not be significantly different from control exposure levels encountered when the phone is switched off.

There is no description in the paper for control for noise, heat, magnetic field or vibration; though magnetic field and heat at the distance at which RF exposure took place are unlikely to be significant, noise (noise from the battery and power circuitry rather than the phone's loudspeaker, which had been silenced) may be. The authors do not describe what type of phone (3G, GSM, CDMA) was used in the study but any of these may be a source of noise audible to a mouse, with GSM arguably being the most audible to a human.

No attempt was made to standardise litter size, or to equate the sex ratio of the litters, which is common practise in teratological studies. It also appears that both male and female offspring were used, and no attempt was made to separate the results by sex.

The tests applied to the animals seem appropriate and both the exposed and control animals were treated the same. However, it is not clear how many animals were used in each part of the

behavioural experiments, only total numbers are given for each complete test. After estimating numbers of offspring likely to be produced by about 40 pregnant female CD-1 mice, some reuse of animals seems likely, so that not all animals would have been naïve during the subsequent tests. This could have affected the results.

No explanation is provided as why the particular age at testing was chosen and why the electrophysiology was performed on much younger animals than those used in the behavioural tests. The lack of statistical tests between treatments at specific ages in the behavioural tests is also of concern.

The most intriguing result, however, is the dose-dependent change in mEPSCs frequency reported in pyramidal neurons. This implies that the exposures were well controlled, stable and relatively reproducible. However, this precision seems highly implausible using cellular phones as exposure systems for free-moving animals. Although use of phones per day has been used as a surrogate for exposure in epidemiology studies, it is a very imprecise and crude measure of exposure that is only used in the absence of a more appropriate metric.

This suggests that another factor in the environment may have had an influence on the behaviour of exposed animals, although it is not possible to identify what this may have been. This part of the study did not have the correct double-blinding. Even though the observers for the behavioural tests were unaware of whether the animals were exposed or not, the animal handlers did know their exposure status, allowing the possibility of unconscious differences in how animals from each group were treated. A suitable exposure system should have operated so that none of the researchers knew the exposure status of the animals; with a coding regime that was only broken after the results of individual tests had been obtained.

Conclusions: The study reports that prenatal exposure to the RF fields from a cellular phone has long lasting detrimental effects on the development of mice, with repeatable and consistent changes observed in behaviour and in electrophysiology. Further, changes in electrophysiology were seen that depended on the number of hours the animals were exposed each day.

However, the simple exposure system in this study consisted of cellular phones, and the RF fields from these phones were at an unstable and unknown level. The quoted SAR of 1.6 W/kg is a value that would be measured in a phantom human head in contact with the phone. The lack of power control, the distance of the animals from the phone and the low absorption cross section of mice in the frequency range 900-1900 MHz together suggest that there may be no significant difference in exposure between some or all of the exposed and control animals. Having many animals in each cage further complicates assessment of the actual exposures.

In summary, it seems implausible that these exposures could have caused the effects reported. If not the RF fields, what then could have been responsible? Because the simple exposure system did not allow it, the exposure status of the animals was not subject to the usual double-blinding, allowing the possibility of unconscious bias in, for example, animal handling. Heating and magnetic fields from the phone were likely to have been insignificant at the locations of the animals, but noise from the battery and power components may have been audible to the exposed group of mice, but not the controls. But it is uncertain whether either of these possibilities could provide a more parsimonious explanation of the results. Overall, the results are intriguing but the lack of control of exposure renders this more like an epidemiological study performed with mice.